Original Article

The Role of Antimullerian Hormone in the Diagnosis of Poly Cystic Ovarian Syndrom.

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Summary:

Background: Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting up to 10% of women of reproductive age. The cardinal features of PCOS are hyperandrogenism (HA) and oligo-anovulation. Many work teams recently have relate the severity of PCOS with Anti mullerian hormone (AMH) or antral follicle count (AFC). Objective: 1) to confirm if there is an increase of serum AMH in our group of patients with PCOS, 2) to relate the AMH level to the follicle status at ultrasound (U/S) in our group of patients, and 3) to search if AMH or AFC can serve as surrogate for the definition of PCOS.

Patients and methods: Twenty five (control) and another 45 participants (with PCOS) were selected for this study. The control women had a mean age of 32.5 year (yr) and the patients group women had a mean age of 28.4 yr. Blood samples were collected from all participants, anthropometric measurements were calculated, and transvaginal U/S was performed to measure the AFC during the early follicular phase. The blood samples were assayed for AMH, follicle-stimulating hormone (FSH), Luteinizing Hormone (LH) and estradiol (E2) and total testosterone (TT).

Results: Basal serum hormonal levels of LH, AMH and TT as well as AFC were significantly higher in the study group than in controls. While basal serum levels of E2 and FSH show no significant difference between the two groups. Body mass index (BMI) is significantly higher in PCOS patients. Basal serum levels of AMH show significant positive correlation with both AFC and basal serum TT levels.The area under the receiver operating characteristic curve (ROCAUC) for AMH and AFC show significant sensitivity and specificity.

Conclusion: serum AMH and AFC appear as a sensitive and specific parameter that would probably help in the diagnosis of PCOS. Both criteria need to be incorporated into the Rotterdam definition for PCOS, and it indicates PCOS only if associated with HA and/or oligo-anovulation.

Key words: Antimullerian hormone(AMH) , antral follicle count(AFC) ,Poly Cystic Ovarian Syndrom(PCOS).

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Introduction:

Polycystic ovary syndrome (PCOS) is a common endocrine disorder, affecting up to 10% of women of reproductive age (1). Its prevalence varies according to the definition used and to the reference population (2).The cardinal features of PCOS are hyperandrogenism (HA) and oligo-anovulation. The metabolic abnormalities often associated with this syndrome (obesity, insulin resistance, hyperinsulinemia and dyslipidemia) are not included in the definition of the syndrome because it is still unclear whether they are intrinsic to the disease or not (3). The current diagnostic classifications as suggested in the Rotterdam conference sponsored by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine (ESHRE/ASRM) (4), use HA, oligo-anovulation and polycystic ovarian morphology (PCOM) at ultrasound. Dewailly and his team (5) reported that in fact the presence of PCOM (defined largely by an excess of follicles, 10 mm at ovarianU/S turned out because PCOM itself is a sign of HA. Anti-Mu¨llerian hormone (AMH) a peptide produced by the granulosa cells (GC) of follicles is highly correlated to the excess of number of follicles in patients with PCOS (6, 7, 8). Many work teams recently have relate the severity of PCOS with AFC or AMH (9, 10). The aim of the present study: 1) to confirm if there is an increase of serum AMH in our group of patients with PCOS, 2) to relate the AMH level to the follicle status at U/S in this group, and 3) to search if AMH or AFC can serve as surrogate for the definition of PCOS.

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Patients and Methods:

This study was performed at Al- Amal clinic for infertility diagnosis and treatment, in Babylon province/Iraq, from November 2012 - May 2013. The main clinical, hormonal and ultrasound data of controls and patients with PCOS are presented in Table 1. Controls: The control population consisted of 25 healthy women (mean age, 32.5 yr, ranging (28.02 -35. 22yr). Their mean BMI was 23.12 kg/m2 ranging 22-25Kg/m2. They attend the clinic because of tubal and/or male infertility. Exclusion criteria were a history of menstrual disturbances (i.e. cycle length either 25 days or 35 days), hirsutism, abnormal serum level of

prolactin or androgens (i.e. serum prolactin > 14 ng/ml on two subsequent determinations, TT > 3.4 mmol/l), PCOM at U/S, and hormonal treatment during the 3 months before the study. None had any components of the Rotterdam classification of PCOS (4). Women with PCOS: Forty-five women were recruited for this study. Mean patients' age was 28.40 yr (ranging from 20-41 yr). Mean BMI was 27.98 kg/m2 (ranging from 22-36 kg/m2). They attend our clinic for HA and/or oligoanovulation and/or infertility due to sperm and/ or tubal abnormality, patient with endometriosis or unexplained infertility were excluded.At least two of the following three items were required for patients' inclusion in this study, according to the Rotterdam classification:

1) HA, defined clinically as the presence of hirsutism (modified Ferriman and Gallwey score >6) (11) and/or biologically by serum TT level greater than 3.4mmol/l, or minor signs such as acne or seborrhea, and/or testosterone > 3.4mmol/l (12).

2) Menstrual and/or ovulatory disturbances, mainly oligomenorrhea i.e. fewer than eight menstrual bleeds over the previous year) and amenorrhea (i.e. no menstrual bleed over the previous 3 months).

3) Presence of polycystic ovary at U/S (PCOM), according to the Rotterdam criteria 12 or more follicles measuring 2-9 mm in diameter.

Investigations: Clinical, hormonal and ultrasound examination were performed in the early follicular phase of the menstrual cycle (i.e. between cycle day 2- cycle day 5). Height and weight measurement was performed. Blood sampling was performed both in PCOS patients and control women. In PCOS patients, the last menstrual period was either spontaneous or induced by the administration of progesterone injection (50 mg/ ampule as single injection). Prolactin, thyroid function test, LH, FSH, E2 and TT levels were measured by Mini Vitic Immuno Diagnostic Assay System (mini VIDAS) method. Subsequently AMH sera levels were measured with AMH\Gen II analysis kit (Beckman coulter, USA) using Enzyme Linked ImmunoSorbant Analysis (ELISA). Ultrasound was performed cycle day 2 by radiology specialist using real time ultrasound device (Philips 11*E), using vaginal probe (7 MHZ) , Follicles measuring 2-9 mm were counted from the lateral to medial margin of each ovary to determine the antral follicle cohort count(AFC). The total number of the follicles per patient counted in both ovaries was used for calculation. Control population have AFC <12 in the range of 2-9 mm in diameter in each ovary at U/S. Patient population has more than 12 follicles in the 2- to 9-mm range in each ovary at U/S.

Statistical methods: Descriptive statistics were expressed as mean and standard deviation (SD). Student's t-test was used to compare groups. Significant relationships between AMH and the AFC and basal serum TT were evaluated by Pearson's correlation coefficient. P- Values < 0.05 were considered to be significant. Receiver operating characteristic curve (ROC) was applied for comparing area under the curve (AUC) for AMH and AFC performance in detecting patients with PCOS (13).Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 17.

Results:

Table 1: Demographic characters of controls and study group. (Values are mean ± SD).

Parameter	Control (n=25)	Patient (n=45)	P value
Age (year)	32.50±7.40	28.40±5.80	p>0.05
BMI (kg/m2)	23.12±3.46	27.98±3.63	p<0.05*
E2 (Pg/ml)	1.82±15.79	45.86±18.61	p>0.05
FSH (mIu/ml)	4.71±1.35	4.32±2.7	P>0.05
LH (mIu/ml)	3.7±1.75	6.59±1.63	p<0.05*
AMH (ng/ml)	2.45±1.48	6.89±5.11	p<0.05*
AFC	7.3±1.87	14.00±1.79	p<0.05*
TT (mmol/l)	2.5±1.65	4.56±1.19	p<0.05*

*Significantly different from corresponding value

Table (1): shows patients and controls characteristics, basal serum levels of E2, FSH, LH, AMH, TT and AFC.There were no significant difference in age between controls and patient's group (32.50 ± 7.40 vs 28.40 ± 5.80 years), but there were significant increase in BMI in the patient's group ($p<0.05$). Serum E2 level in CD2 shows no significant difference between these two group ($p>0.05$), the same is applied to mean basal serum levels of FSH (4.71 ± 1.35 vs 4 .32 ± 2.7mIu/ml), Serum LH level was significantly higher in PCOS group ($p<0.05$). A significantly higher mean rank of AMH in PCOS group than in control (2.45 ± 1.48 vs 6.89 ± 5.11 ng/ml) ($p<0.05$).

As table (1) demonstrate mean serum TT is significantly higher in PCOS group compared to control ($p<0.05$) (2.5 ± 1.65 vs 4.56 ± 1.19 mmol/l), the same can be said in AFC which was significantly higher in PCOS group (7.3 ± 1.87 vs 14.00 ± 1.79).($P<0.05$).

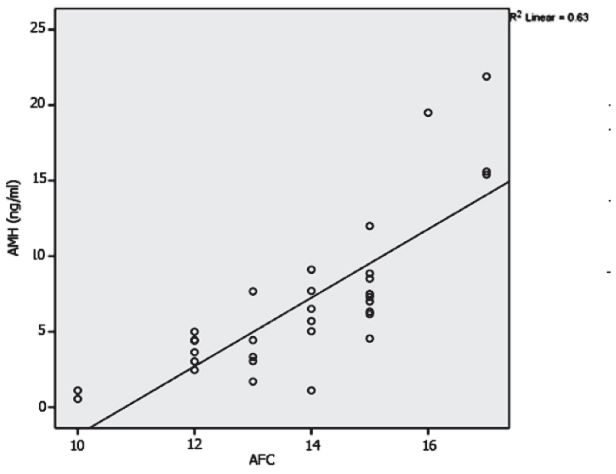

Figure (1): Correlation of Antimullerian hormone (AMH) with Antral follicle count (AFC) using Pearson's correlation coefficient. r= 0.798, significant positive correlation, P < 0.05.

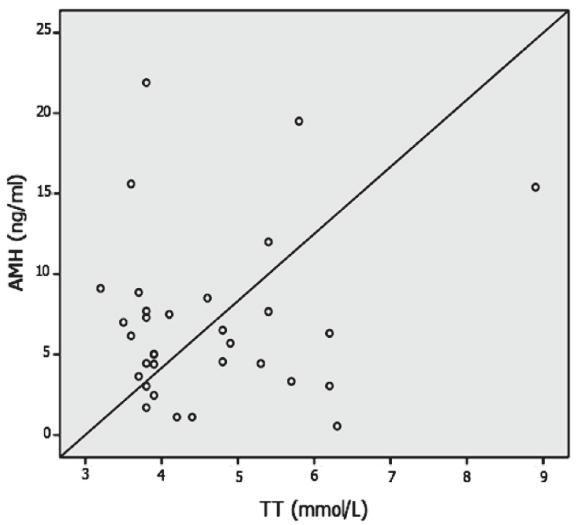

Figure (2): Correlation of basal Antimullerian hormone (AMH) and Testosterone (TT) using Pearson's correlation coefficient. r=0 .742, significant positive correlation, p<0.05.

Figure (1): demonstrate that there was highly significant correlation between the values of AMH and AFC in the study population r= 0.798, P < 0.05.

Figure (2): Shows the significant positive correlation between basal mean AMH and TT (r=0.742, p<0.05).

When the ROC analysis was restricted to -PCOS patients, the AUC for AMH was 0.854, with the best compromise at 6 ng/ml (Sensitivity 88%, Specificity 90%). The AUC for AFC was 0.800 with best compromise at 14 (sen of 84 % and spe 90%). As shown in table 2.

Table 2: different threshold values, sensitivities and specificities when applying ROC for AMH and AFC in PCOS patients.

Parameter	Threshold	Sensitivity %	Specificity %
Serum AMH	4	80	88
	5	88	86
	6	88	90
Follicle number	12	81	83
	14	84	90
	16	75	80

Discussion:

The diagnosis of PCOS using The Rotterdam ESHRE/ ASRM-Sponsored PCOS consensus workshop group have been revised by many workers in this field , concentrating on the threshold of AFC and trying to have new indicator for the diagnosis and follow up of treatment of this syndrome. Herein we tried to study the role of AMH and AFC in the diagnosis of this syndrome. We selected a 25 control women and 45 women with PCOS patients for the analysis whom clinical data are listed in table 1. There were no significant difference in age between controls and patients. Some workers demonstrate that the prevalence of PCOS by the Rotterdam AFC criterion is significantly impacted by age, and there is a fall in AFC with age among both ovulatory (14,15) and PCOS women (16).Murphy and coworkers (17) showed that half of women with PCO at a mean age of 30 no longer display this finding 8 years later.

Our findings are consistent with previous studies (7, 18, 5) that found a greater BMI in women with PCOS than in women with normal ovaries .Obesity or overweight have negative impact on the consequences of PCOS (18); Obesity is present in 30–75% of women with the syndrome mostly associated with insulin resistance and hyperandrogenaemia (19). The insignificant difference in basal serum levels of E2 and FSH between the control and the study population (table 1) have been demonstrated by previous studeis (6, 5). Our study confirmed previous studies (7, 5, 9) that found basal serum levels of LH were significantly higher in PCOS patients than in control (Table 1), and altered gonadotropin dynamics is another key feature of PCOS. Although a higher LH level drives the ovarian theca cells to produce more androgens, FSH may be the more immediate cause of anovulation and several studies have demonstrated that LH value or LH/FSH ratio is not helpful in establishing this diagnosis in such patients (20), on the other hand Homburg et al.10) concluded that LH above 6 IU/l have diagnosed 82.6% of women with PCOS. Our study demonstrates that basal serum AMH is significantly higher in PCOS group than in control. In human ovaries, AMH is produced by GC from 36 weeks of gestation until menopause, with the

highest expression being in small antral follicles, (21). AMH production gradually declines as follicles grow; once follicles reach a size at which they are dominant, it has largely disappeared. Cook and coworkers in 2002 (22) found higher levels of AMH in their PCOS population which were associated with lower values of FSH levels (as in our study) but with lower E2 levels (in the former).Understanding the reason for the raised AMH in PCOS may give clues as to the mechanism of anovulation as AMH appears to have a major inhibitory role during folliculogenesis, which may contribute to anovulation in PCOS. Experimental data carried out on cultured GCs demonstrated that AMH inhibits the conversion rate of androgens to E2 by down-regulating the aromatase gene expression, this inhibiting effect of AMH on aromatase activity acts through a decrease in GC sensitivity to FSH (21). In normal ovulatory cycle there is a balance between the opposite effects of AMH and FSH on aromatase activity this might be crucial for the cohort of the follicles, at the time of the selection process. This would allow aromatase to escape from AMH inhibition, thus conferring to the selected follicles the ability to secrete E2. Such a phenomenon could be altered in PCOS; this supports the physiological relevance of the inverse relationship between AMH and E2, which has been found in PCOS women (22) or in non-PCOS patients (23). Many workers (6, 24) agreed with our result regarding the AFC, there were significant increase in the count in PCOS patients in comparism with the control group (7.3 ±1.87vs 14.00 ± 1.79). Some research work as that of Dewailly and colleagues in 2011 (9) have increased the threshold number of follicle involved in the diagnosis of PCOS in Rotterdam criteria to 14 or even 16.In our opinion is that the significant increase in the threshold is due to the improvement of the resolving power of U/S images with new appliances, with 2 Dimension U/S or 3 Dimension U/S as well.Since serum TT correlates with AFC in women with PCOS (25), our finding of increased serum TT is consistent with previous studies on this topic (26, 27). We hypothesized that this reflected the promoting effect of intraovarian androgens on follicle growth.

There were significant positive correlation between basal serum AMH levels and AFC in our study population($r= 0.798$), $p< 0.05$, (Figure 1), Dewailly and his coworkers (9) concluded the same results. The increase in AMH concentration is largely due to the increase in production of AMH by each follicle and not just a consequence of an increase in follicles number (28).There were significant positive correlation between basal serum levels AMH and TT, r=0 .742, this result ids in agreement with Homburg work (29) .This significant relationship between AMH and androgens that seems specific to PCOS because it has not been found in controls in a study done by Pigny and his team in 2003 (6). For many years the excess in intraovarian androgens has been suspected to disturb folliculogenesis, through a proatretic effect on growing follicles especially in small follicles whose GCs are the richest in androgen receptors (30).In this study, the diagnostic performance of AMH and AFC in the diagnosis of PCOS was assessed using the ROC curves. The AUC of serum AMH assay yielded a satisfying value of 0.854, higher however than the one obtained with the 2- to 9-mm follicle count (0.800) which was significantly not different by using Wilcoxon Signed Ranks Test . With a cutoff value of 6 ng/ml, for AMH level and 14 for AFC with acceptable sensitivity and specificity(table 3) for both in PCOS patients as defined by the Rotterdam criteria, the follicle count appears as good as diagnostic tool as AMH measurement, at least in our hands. This finding is different than that of pigny and his team (7) who found that AFC is better than AMH in diagnosis of PCOS patients. However, it must be stressed that comparing both studies is somewhat artificial because the latter data were obtained in a different series of patients, using different inclusion criteria. Whatsoever, it can be proposed from the present data to perform an AMH assay in medical centers where the U/S count of small antral follicles is not available. In conclusion, we agree with some of recent studies (5, 31, 10) that for the definition of PCOM and PCOS, serum AMH appears as a sensitive and specific parameter that would probably be easier to reproduce from one to another center than the follicle count, as the latter is highly dependent on the evolving quality of the machines and/or the operator skill. Indeed the single measurement of AMH in the early follicular phase appeared to be a valuable surrogate for the AFC IN PCOS and it could therefore be used in place of the U/S data in the Rotterdam definition. However, it must be kept in mind that neither a high value of AMH nor a high AFC in PCOS is per se sufficient to ascertain PCOS. Both criteria need to be incorporated into the Rotterdam definition, and it indicates PCOS only if associated with HA and/or oligo-anovulation. This emphasizes the need for a careful exclusion diagnosis lying mainly on clinical history, before using the Rotterdam definition including either the AFC or the serum AMH level.

Lastly, we must recognize that our control group included exclusively patients referred to our clinic. It might therefore be not fully representative of the general population. Further studies are required to validate our data in other settings.

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